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Christine Todd Whitman, Administrator
US Environmental Protection Agency
Ariel Rios Building
Room 3000, #1101-A
1200 Pennsylvania Avenue, NW
Washington, DC 20460

Subject: Comments on the HPV test plan for propargyl alcohol

Dear Administrator Whitman,

The following are comments on the test plan for propargyl alcohol (CAS no. 616-45-4) for the HPV program, submitted by the BPPB Consortium on behalf of the Propargyl Alcohol Consortium ("the Consortium"). These comments are submitted on behalf of People for the Ethical Treatment of Animals (PETA), the Physicians Committee for Responsible Medicine (PCRM), the Humane Society of the United States, the Doris Day Animal League, and Earth Island Institute. These animal, health and environmental protection organizations have a combined membership of more than ten million Americans.

The Consortium proposes conducting a combined repeat-dose, reproductive and developmental toxicity test (OECD no. 421) on propargyl alcohol. This test will kill at least 675 mammals.

The Consortium should note that an *in vitro* developmental toxicity test, the rodent embryonic stem cell test, is available and suited for a screening level program such as the HPV chemical-testing program. This test has recently become commercially available in the U.S., and last year it was validated by the European Centre for the Validation of Alternative Methods, after which the Centre's Scientific Advisory Committee concluded that it was ready to be considered for regulatory purposes (Genschow 2002). We therefore urge the Consortium to keep abreast of progress in this field, and to consider the use of this validated, commercially available and inexpensive non-animal test. We advise the Consortium to correspond directly with the EPA about this issue. We also hope that the Consortium will feel free to contact us for advice about the laboratories that are currently conducting this test. One objection that may be made to the use of the embryonic stem cell test is that it provides data only on developmental toxicity, whereas the proposed test would generate data on reproductive and subchronic toxicity as well. However, it should be noted that the test plan states that sufficient data already exist for subchronic and reproductive toxicitye (pp. 13, 15).

An additional concern with the testing proposal is that the toxicity of propargyl alcohol is likely to show such high interspecies variability that data generated by additional animal studies will have little relevance to humans. The basis for this statement is the fact that toxicity due to unsaturated alcohols (e.g. propargyl alcohol) is not usually caused by the alcohols themselves, but by one or more of their metabolites (DeMaster 1994), and propargyl alcohol metabolism differs markedly between species. The marked interspecies variability of propargyl alcohol

metabolism has been shown in studies on rats and mice (Banijamali 1999, 2000, Dix 2001). In rats, propargyl alcohol (2-propyn-1-ol) was found to be oxidized to the aldehyde, 2-propyn-1-al, which then undergoes either oxidation to 2-propyn-1-oic acid, or glutathione conjugation in combination with reduction, and these processes are followed by a range of further metabolic steps, resulting in the urinary excretion of at least five different end-products (Banijamali 1999). In mice, on the other hand, although some of the propargyl alcohol is oxidized to 2-propyn-1-al, some undergoes glucuronide conjugation. In addition, more of the 2-propyn-1-al undergoes glutathione conjugation than in mice, with only a small proportion being oxidized to 2-propyn-1-oic acid. Furthermore, the excreted end-products of the glutathione conjugation pathway are different from those in rats (Banijamali 2000). Most of these interspecies differences are likely to affect toxicity, as much of unsaturated alcohol toxicity is directly due to the aldehyde (DeMaster 1994), more of which is formed in rats, and other factors affecting toxicity include the release of free oxygen radicals (during reduction, for example), and depletion of glutathione by conjugation. It hardly needs to be mentioned that rats and mice are far more closely related to each other than to humans, so one would expect their interspecies differences to be far less than with humans.

The simple fact that the metabolism of propargyl alcohol is so complex, with multiply branched pathways, supports the existence of marked interspecies variability, as the relative importance of the different pathways is unlikely to be consistent between taxa. This complexity applies even to what is considered to be the most well-known metabolic step, the oxidation of propargyl alcohol to 2-propyn-1-al. It used to be assumed that the enzyme responsible for this oxidation is alcohol dehydrogenase, but recent studies have suggested that a considerable range of other catalysts are involved, including liver catalase (DeMaster 1994), CYP 2E1 (a microsomal cytochrome; Morgan 1982, Albano 1991, Moridani 2001), and chloroperoxidase (Hu 1998), in addition to free hydroxyl radicals (Cederbaum 1981).

A final important point is that a developmental toxicity study on propargyl alcohol has in fact been conducted previously, but is not mentioned in the test plan. This study was carried out on a frog, *Xenopus laevis*, and the compound was found to be moderately teratogenic, with a mortality/malformation index of 3.4 (Dawson 1990).

To conclude, animal studies are unlikely to provide any useful information about the human developmental toxicity of propargyl alcohol. The question remains as to how relevant information can be obtained, and we suggest three approaches:

(i) *The embryonic stem cell test*: This is superior to *in vivo* tests because stem cells have fewer species-specific characteristics than whole animals. All known propargyl alcohol metabolites should be tested.

(ii) *An exposure and epidemiology study*. The test plan provides little information about the human exposure to propargyl alcohol (pp. 6-7), and far more detail should be included in this section. More than 54,000 people per year are occupationally exposed to propargyl alcohol in the USA (NIOSH), and an epidemiology study is therefore feasible. The exposed population includes nearly 20,000 women, so an epidemiology study could include an investigation of developmental and reproductive toxicity.

(iii) *Analysis of human metabolism.* Urine should be collected from persons who have ingested propargyl alcohol (accidentally or as suicide attempts, for example). This would enable analysis of the metabolites, to determine whether they show any similarity to those in rat and/or mouse urine. A discussion of human toxicity on the basis of rodent toxicity is meaningless without this information.

Thank you for your attention to these comments. We can be reached via e-mail at RichardT@PETA.org.

Sincerely,

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Federal Agency Liaison
People for the Ethical Treatment of Animals

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